Fig. 1

```
  1 ATG GCT GGA CCT GCC ACC CAG AGC CCC ATG AAG CTG ATG GCC CTG  45
 46 CAG CTG CTG CTG TGG AGT GCA CTC TGG ACA GTG CAG GAA GCC ACC  90
 91 CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG AGC TTC CTG CTC AAG 135
136 TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC 180
181 CAG GAG AAG CTG GCA GGC TGC TTG AGC CAA CTC CAT AGC GGC CTT 225
226 TTC CTC TAC CAG GGG CTC CTG CAG GCC CTG GAA GGG ATC TCC CCC 270
271 GAG TTG GGT CCC ACC TTG GAC ACA CTG CAG CTG GAC GTC GCC GAC 315
316 TTT GCC ACC ACC ATC TGG CAG CAG ATG GAA GAA CTG GGA ATG GCC 360
361 CCT GCC CTG CAG CCC ACC CAG GGT GCC ATG CCG GCC TTC GCC TCT 405
406 GCT TTC CAG CGC CGG GCA GGA GGG GTC CTA GTT GCC TCC CAT CTG 450
451 CAG AGC TTC CTG GAG GTG TCG TAC CGC GTT CTA CGC CAC CTT GCC 495
496 CAG CCC TAA TAA                                          508
```

stop codon (see: SEQ ID NO: 17)

Fig. 2

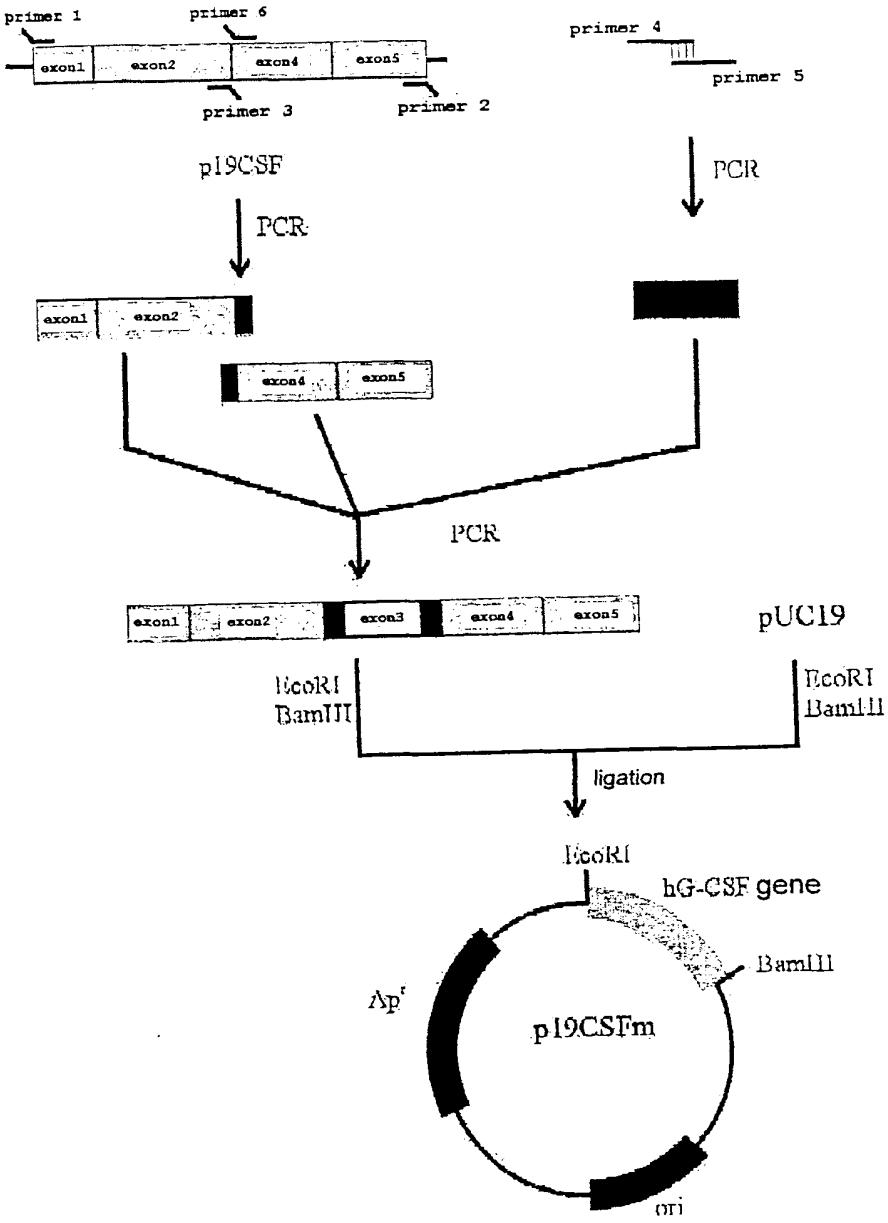

primer 1
primer 6
exon1
exon2
exon4
exon5
primer 3
primer 2
primer 4
primer 5
p19CSF
PCR
PCR
exon1
exon2
exon4
exon5
PCR
exon1
exon2
exon3
exon4
exon5
pUC19
EcoRI
BamHI
EcoRI
BamHI
ligation
EcoRI
hG-CSF gene
BamHI
Ap
p19CSFm
ori

Fig. 3

```
                -30                                                     -16
  1 ATG GCT GGA CCT GCC ACC CAG AGC CCC ATG AAG CTG ATG GCC CTG  45

                -15                                          -1  +1
 46 CAG CTG CTG CTG TGG AGT GCA CTC TGG ACA GTG CAG GAA GCC ACC  90
                                                             Thr

                  2                                                  16
 91 CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG AGC TTC CTG CTC AAG 135
    Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys

                 17                                                  31
136 TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA GCG CTC 180
    Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu

                 32                                                  46
181 CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC GAG GAG 225
    Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu

                 47                                                  61
226 CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT CCC CTG 270
    Leu Val Leu Leu GLy His Ser Leu Gly Ile Pro Trp Ala Pro Leu

                 62                                                  76
271 AGC AGC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC TTG AGC 315
    Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser

                 77                                                  91
316 CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG CAG GCC 360
    Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala

                 92                                                 106
361 CTG GAA GGG ATC TCC CCC GAG TTG GGT CCC ACC TTG GAC ACA CTG 405
    Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu

                107                                                 121
406 CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG CAG ATG 450
    Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met

                122                                                 136
451 GAA GAA CTG GGA ATG GCC CCT GGC CTG CAG CCC ACC CAG GGT GCC 495
    Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala

                137                                                 151
496 ATG CCG GCC TTC GGC TCT GCT TTC CAG CGC CGG GCA GGA GGG GTC 540
    Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val

                152                                                 166
541 CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG TAC CGC 585
    Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg

                167                 174
586 GTT CTA CGC CAC CTT GCC CAG CCC TAA TAA                     616
    Val Leu Arg His Leu Ala Gln Pro stop codon
```

(see: SEQ ID NO: 18)

(see: SEQ ID NO: 19)

Fig. 4

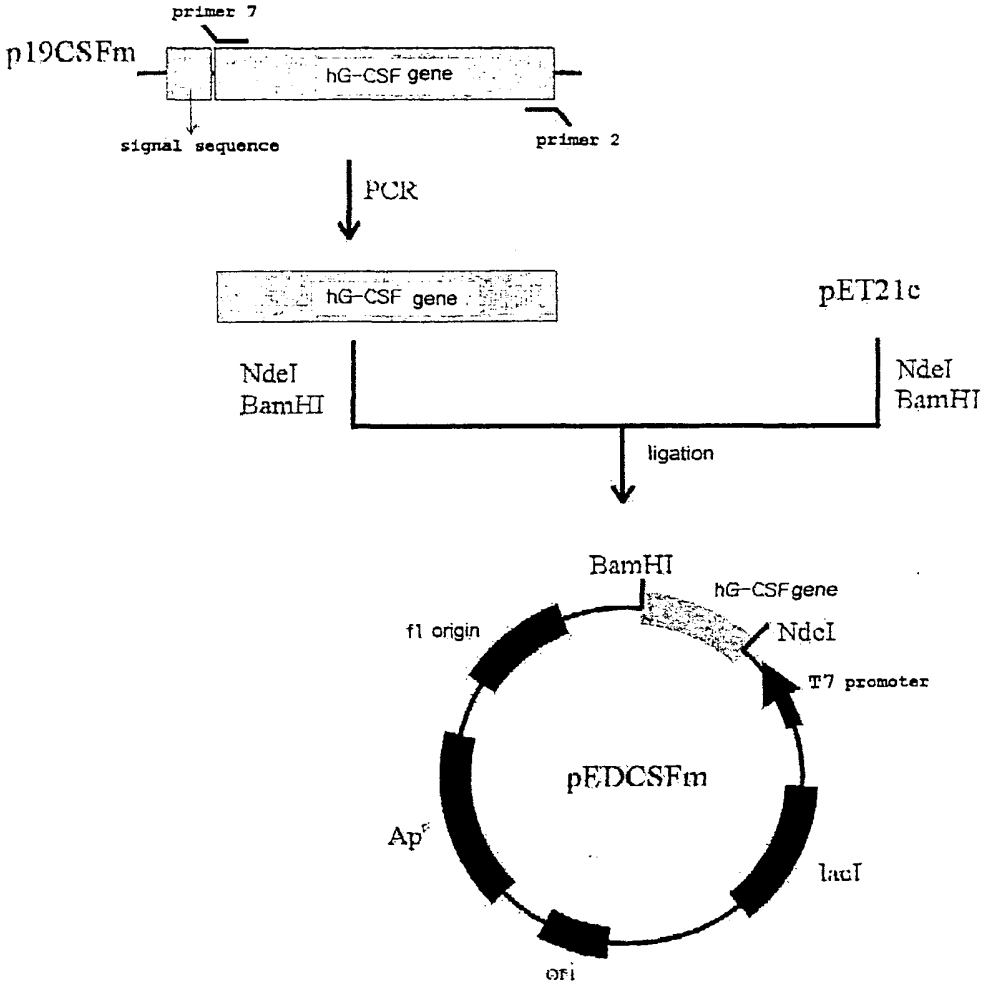

primer 7
p19CSFm
hG-CSF gene
signal sequence
primer 2
PCR
hG-CSF gene
pET21c
NdeI
BamHI
NdeI
BamHI
ligation
BamHI
hG-CSFgene
f1 origin
NdeI
T7 promoter
pEDCSFm
Ap
lacI
ori

Fig. 5

```
    1                                                              15
  1 ATG ACC CCC CTG GGC CCT GCC AGC TCC CTG CCC CAG AGC TTC CTG   45
    Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu

   16                                                              30
 46 CTC AAG TGC TTA GAG CAA GTG AGG AAG ATC CAG GGC GAT GGC GCA   90
    Leu Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala

   31                                                              45
 91 GCG CTC CAG GAG AAG CTG TGT GCC ACC TAC AAG CTG TGC CAC CCC  135
    Ala Leu Gln Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro

   46                                                              60
136 GAG GAG CTG GTG CTG CTC GGA CAC TCT CTG GGC ATC CCC TGG GCT  180
    Glu Glu Leu Val Leu Leu GLy His Ser Leu Gly Ile Pro Trp Ala

   61                                                              75
181 CCC CTG AGC AGC TGC CCC AGC CAG GCC CTG CAG CTG GCA GGC TGC  225
    Pro Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys

   76                                                              90
226 TTG AGC CAA CTC CAT AGC GGC CTT TTC CTC TAC CAG GGG CTC CTG  270
    Leu Ser Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu

   91                                                             105
271 CAG GCC CTG GAA GGG ATC TCC CCC GAG TTG GGT CCC ACC TTG GAC  315
    Gln Ala Leu Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp

  106                                                             120
316 ACA CTG CAG CTG GAC GTC GCC GAC TTT GCC ACC ACC ATC TGG CAG  360
    Thr Leu Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln

  121                                                             135
361 CAG ATG GAA GAA CTG GGA ATG GCC CCT GCC CTG CAG CCC ACC CAG  405
    Gln Met Glu Glu Leu Gly Met Ala Pro Ala Leu Gln Pro Thr Gln

  136                                                             150
406 GGT GCC ATG CCG GCC TTC GCC TCT GCT TTC CAG CGC CGG GCA GGA  450
    Gly Ala Met Pro Ala Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly

  151                                                             165
451 GGG GTC CTA GTT GCC TCC CAT CTG CAG AGC TTC CTG GAG GTG TCG  495
    Gly Val Leu Val Ala Ser His Leu Gln Ser Phe Leu Glu Val Ser

  166                                                  175
496 TAC CGC GTT CTA CGC CAC CTT GCC CAG CCC TAA TAA              531
    Tyr Arg Val Leu Arg His Leu Ala Gln Pro stop codon
```

(see: SEQ ID NO: 20)
(see: SEQ ID NO: 21)

Fig. 6

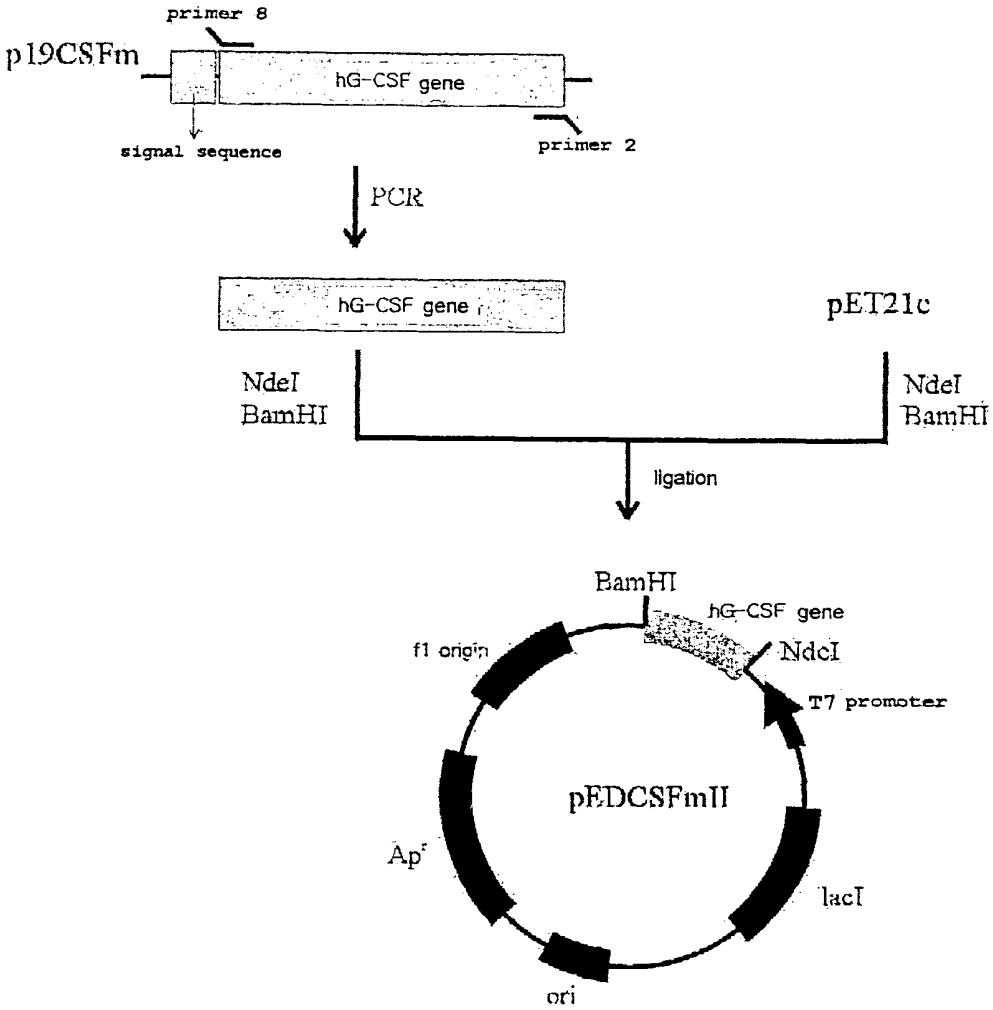

primer 8
p19CSFm
hG-CSF gene
signal sequence
primer 2
PCR
hG-CSF gene
pET21c
NdeI
BamHI
NdeI
BamHI
ligation
BamHI
hG-CSF gene
f1 origin
NdeI
T7 promoter
pEDCSFmII
Ap
lacI
ori

Fig. 7

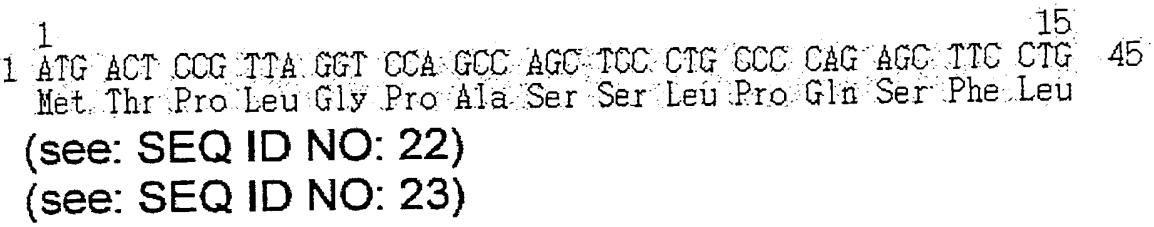

1 15
1 ATG ACT CCG TTA GGT CCA GCC AGC TCC CTG CCC CAG AGC TTC CTG 45
Met Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu
(see: SEQ ID NO: 22)
(see: SEQ ID NO: 23)

Fig. 8

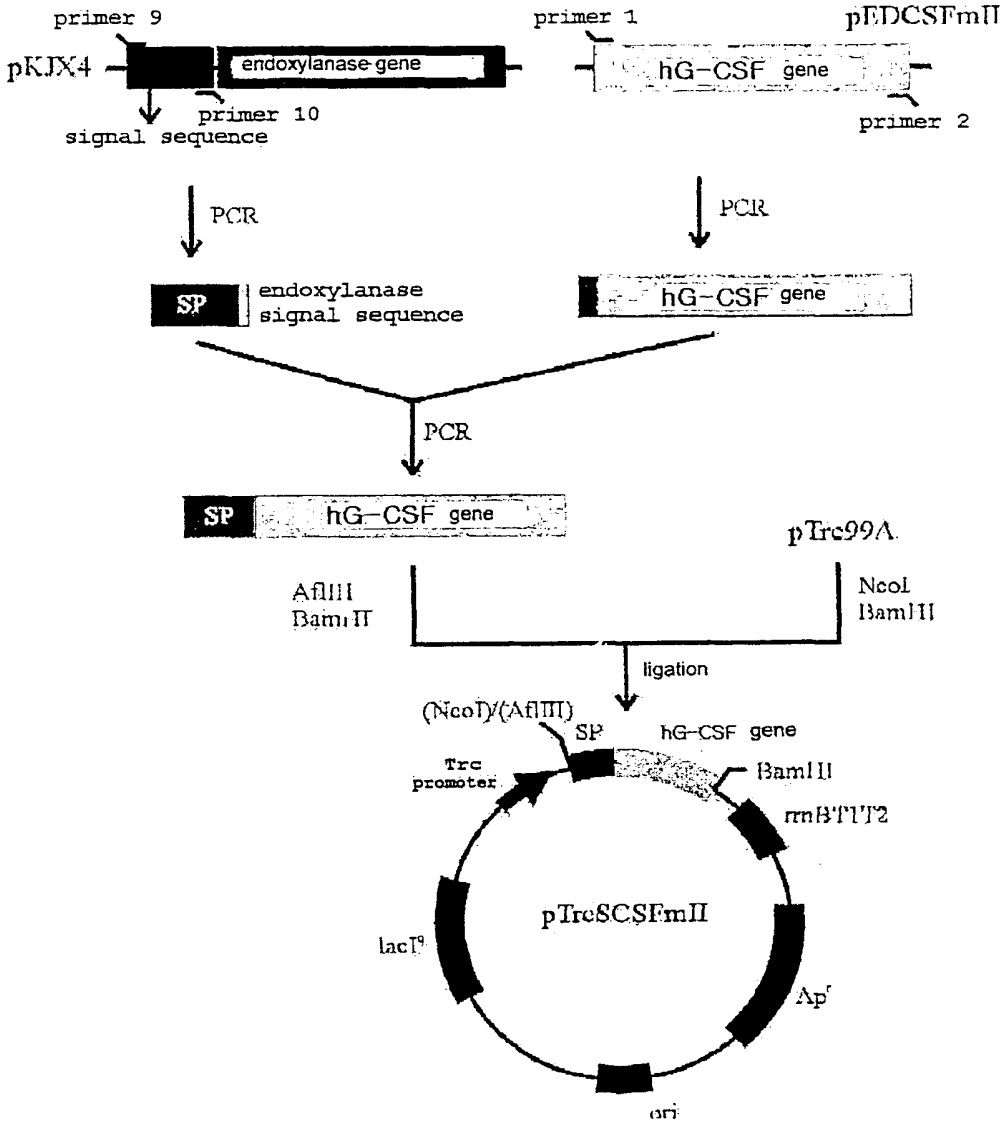


Fig. 9

```
   -28                                                          -14
 1 ATG TTT AAG TTT AAA AAG AAA TTC TTA GTG GGA TTA ACG GCA GCT  45
   Met Phe Lys Phe Lys Lys Lys Phe Leu Val Gly Leu Thr Ala Ala

   -13                                                  -1  +1   2
46 TTC ATG AGT ATC AGC ATG TTT TCT GCA ACC GCC TCT GCA ACT CCG  90
   Phe Met Ser Ile Ser Met Phe Ser Ala Thr Ala Ser Ala Thr Pro

    3                                                           17
91 TTA GGT CCA GCC AGC TCC CTG CCC CAG AGC TTC CTG CTC AAG TGC 135
   Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys
```

(see: SEQ ID NO: 24)
(see: SEQ ID NO: 25)

Fig. 10

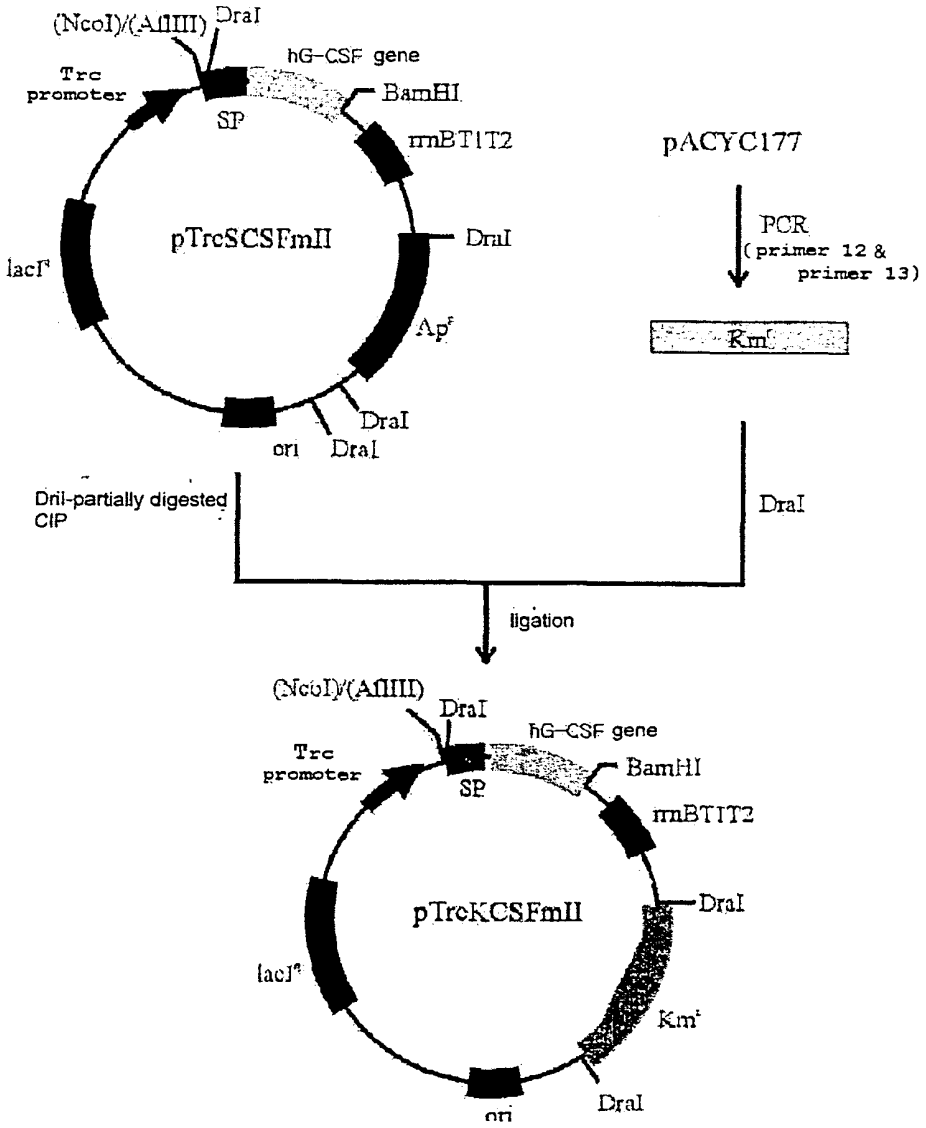


Fig. 11

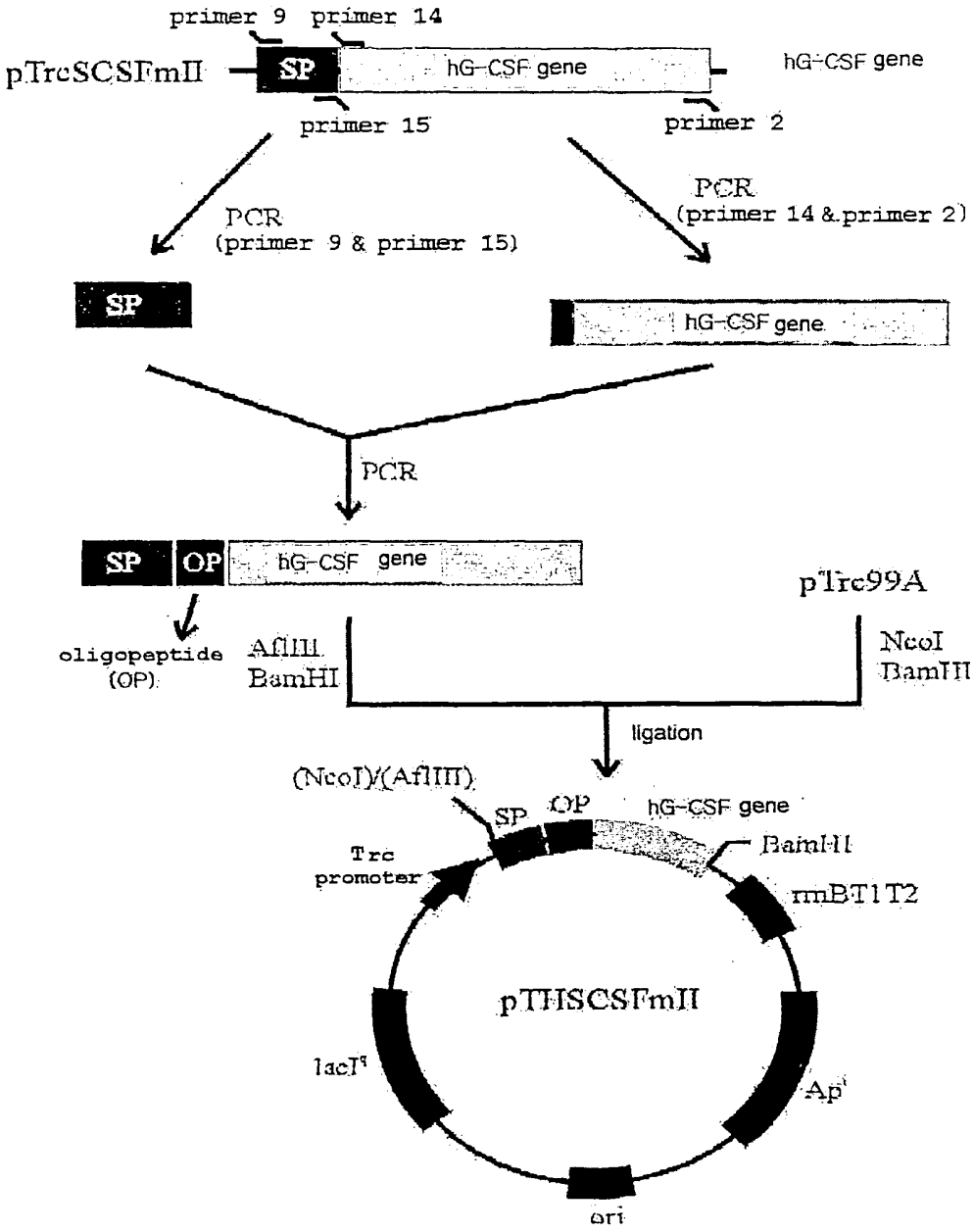
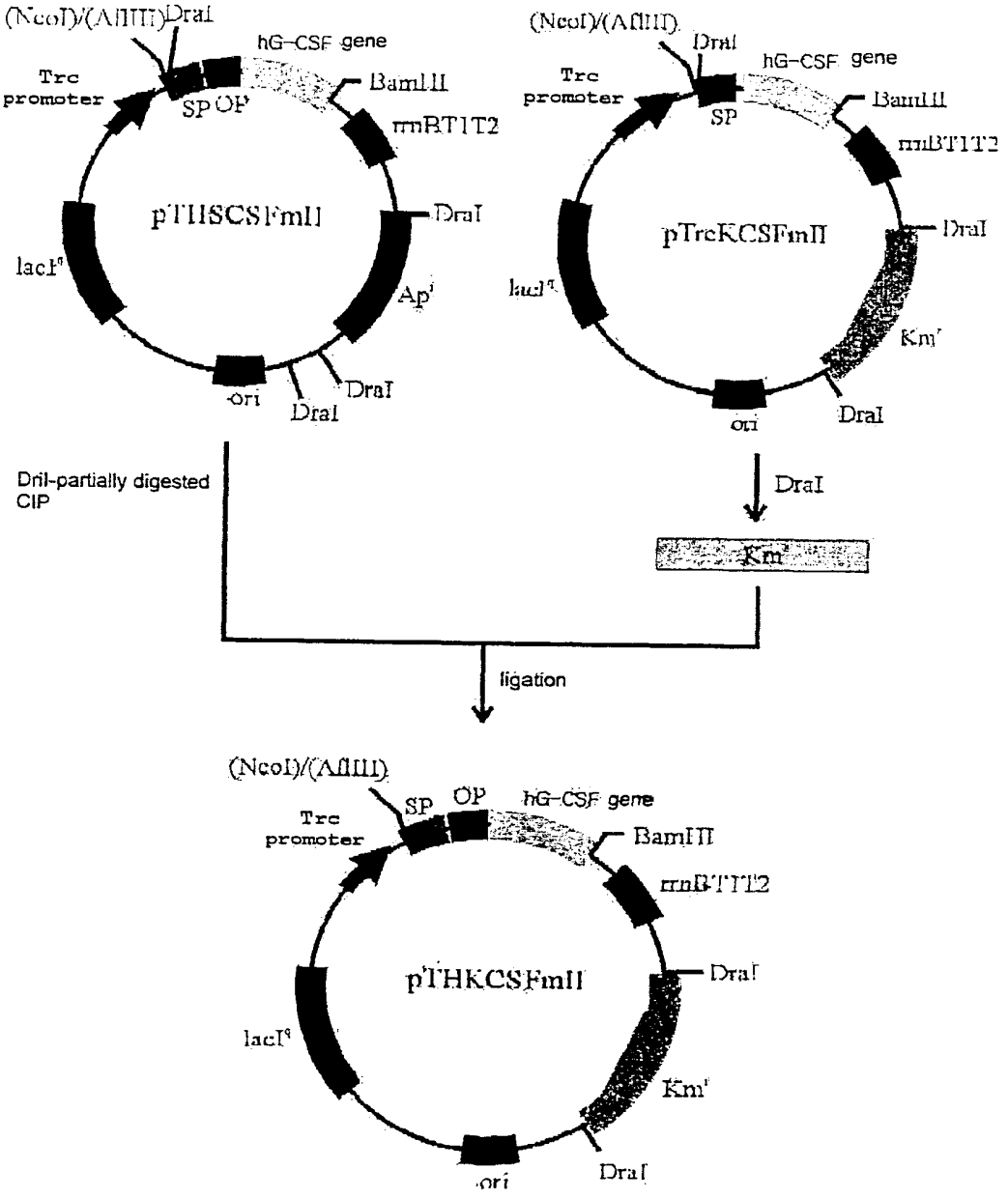

primer 9
primer 14
pTrcSCSFmII
SP
hG-CSF gene
hG-CSF gene
primer 15
primer 2
PCR
(primer 9 & primer 15)
PCR
(primer 14 & primer 2)
SP
hG-CSF gene
PCR
SP
OP
hG-CSF gene
pTrc99A
oligopeptide
(OP)
AflIII
BamHI
NcoI
BamHI
ligation
(NcoI)/(AflIII)
SP
OP
hG-CSF gene
Trc
promoter
BamHI
rrnBT1T2
pTHSCSFmII
lacI
Ap
ori

Fig. 12

```
   -28                                                                -14
 1 ATG TTT AAG TTT AAA AAG AAA TTC TTA GTG GGA TTA ACG GCA GCT  45
   Met Phe Lys Phe Lys Lys Lys Phe Leu Val Gly Leu Thr Ala Ala

   -13                                                  -1  +1   2
46 TTC ATG AGT ATC AGC ATG TTT TCT GCA ACC GCC TCT GCA GCT GGC  90
   Phe Met Ser Ile Ser Met Phe Ser Ala Thr Ala Ser Ala Ala Gly

     3                                                             17
91 CCG CAC CAT CAC CAT CAC CAT ATC GAG GGA AGG ACT CCG TTA GGT 135
   Pro His His His His His His Ile Glu Gly Arg Thr Pro Leu Gly

    18                                                             32
136 CCA GCC AGC TCC CTG CCC CAG AGC TTC CTG CTC AAG TGC TTA GAG 180
    Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys Cys Leu Glu
```

(see: SEQ ID NO: 26)
(see: SEQ ID NO: 27)

Fig. 13


(NcoI)/(AflIII)
DraI
hG-CSF gene
Trc promoter
SP
OP
BamHI
rrnBT1T2
pTHSCSFmII
DraI
lacI^q
Ap^r
ori
DraI
DraI
(NcoI)/(AflIII)
DraI
hG-CSF gene
Trc promoter
SP
BamHI
rrnBT1T2
pTrcKCSFmII
DraI
lacI^q
Km^r
ori
DraI
DraI-partially digested CIP
DraI
Km^r
ligation
(NcoI)/(AflIII)
SP
OP
hG-CSF gene
Trc promoter
BamHI
rrnBT1T2
pTHKCSFmII
DraI
lacI^q
Km^r
ori
DraI